

The minimum protective level of antibodies in smallpox *

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Blood samples from 57 contacts of 6 smallpox cases were tested for haemagglutination-inhibiting (HI) and neutralizing antibodies. All 6 contacts who subsequently developed smallpox were unvaccinated and had neutralizing antibody titres of 10 or less. However, 6 unvaccinated contacts with similar antibody levels did not develop smallpox. None of the 41 vaccinated contacts, regardless of their antibody level, contracted the disease.

As in many other infectious diseases, the minimum level of humoral antibody necessary to prevent smallpox is not known. It is generally thought that a high level of antibody, specially neutralizing antibody, circulating in the blood protects an individual against smallpox, although the disease has been reported to occur in persons with a high level of neutralizing antibody (1). In the present paper, an attempt is made to examine this question. The study is incomplete, but because the rapid disappearance of smallpox from Calcutta and nearby areas has meant that subjects can no longer be found for study, it was thought worth while to document whatever data have been collected, and to try if possible to draw some conclusions. Work of this nature does not appear to have been done previously.

MATERIALS AND METHODS

The procedure followed was to collect blood samples from voluntary donors amongst the contacts of smallpox cases in the Calcutta area, test the samples for haemagglutination-inhibiting (HI) and neutralizing antibodies, and follow up the donors to see if they developed smallpox.

Smallpox patients

Only virologically proved cases were included in the study. The patients were clinically examined, and the presence or absence of vaccination marks was recorded. The smallpox patients whose contacts were studied were designated as "index cases". The criteria for classifying cases in descending order of severity as "haemorrhagic", "confluent" and "discrete" and the definition of the word "contact" were the same as adopted previously (5-7). The presence of a vaccination scar was taken as evidence of previous vaccination. Vaccinations done after the day of our first visit, and histories of previous vaccinations or revaccinations, were not recorded. The day of onset of fever was taken as the date of onset of the disease. The contacts were visited every 3rd or 4th day for 3 weeks to ascertain if any of them developed smallpox. Whether the contacts lived in the same room or in the same compound as the index cases was recorded.

Collection of blood

On the first day of our visit after an index case was traced, blood specimens were collected from contacts. Venous blood was drawn whenever possible. In 15 instances when permission for this was refused, blood was collected on filter paper discs by finger tip puncture (2). The discs were air-dried and preserved at -20°C . Before testing they were soaked in saline overnight in the refrigerator; the fluid was then tested for antibodies. The technique was shown to be satisfactory by simultaneous testing of the venous blood and finger tip blood of the same subjects (3 vaccinated volunteers and 3 smallpox patients).

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HI and neutralizing tests

The techniques were the same as described previously (8). The sensitivity of the neutralizing tests was checked from time to time by incorporating a reference serum obtained from the Statens Seruminstitut, Copenhagen, in the same tests together with a hyper-immune serum prepared locally.

RESULTS

Altogether 57 contacts of 30 index cases (16 discrete, 9 confluent, and 5 haemorrhagic) were studied; 15 of the contacts had no primary vaccination mark, but the others had. The age of the contacts varied from 4 to 70 years; 34 were males and 23 females. Twenty-seven contacts lived in the same room as the index case concerned, and 30 in the same compound. Only 6 of the 57 contacts developed the disease. The details are shown in Table 1, while the HI and neutralizing antibody titres of the 57 contacts are shown graphically in Fig. 1.

DISCUSSION

As the number of contacts studied was rather small, any deductions drawn from the results should be taken as provisional.

All 6 contacts who contracted smallpox were previously unvaccinated, and all had HI antibody titres (expressed as the reciprocal of the highest dilution of serum inhibiting haemagglutination or pock formation) of 20 or less and neutralizing antibody titres of 10 or less. None of the contacts, vaccinated or unvaccinated, with a pre-existing neutralizing titre of 20 or more developed the disease. However, other contacts who were unvaccinated and had antibody levels as low as the 6 who contracted smallpox did not develop the disease. It is worth noting that none of the vaccinated contacts, even without detectable neutralizing antibody, developed smallpox. These findings indicate that the humoral antibody level at the time of infection may not be the only factor in determining whether a person exposed to infection will develop the disease, and that other factors such as tissue immunity may play some role. Secondly, the results show that the rapid formation of antibody after infection and not its initial level in the blood may be an important factor in deciding whether the infecting organism is able to produce clinical illness.

Four of the 6 contacts who developed the disease lived in the same room as an index case, but the other 2 did not, although they lived in the same

compound. It has previously been shown (4) that the infection rate is practically the same among room contacts and compound contacts, because in any case the people mix freely.

Of the 6 secondary cases, 4 contracted the disease from discrete index cases, 1 from a confluent case, and 1 from a haemorrhagic case, suggesting that the mildness of a case does not in any way diminish its ability to spread infection. All the secondary cases were discrete, although the clinical form of the index cases varied. As pointed out previously (5), the expression of clinical illness in an individual depends on the interaction between the virulence of the invading organism and the immunity developed in the individual.

Five of the 6 secondary cases developed the disease 10–12 days after the onset of fever of the index cases, and the other 7 days after. It may be that the short incubation period of the latter case was attributable to a massive inoculum from the index case concerned (haemorrhagic cases are known (6) to excrete a very high titre of virus in the throat) or to his acquiring the infection during the incubation period of the index case (7).

The sera of 2 unvaccinated contacts (serial Nos. 1 and 2) showed a fair level of antibody. The explanation might be that they had earlier had subclinical infections, successful vaccinations without a scar persisting, or mild smallpox without detectable pock marks (3).

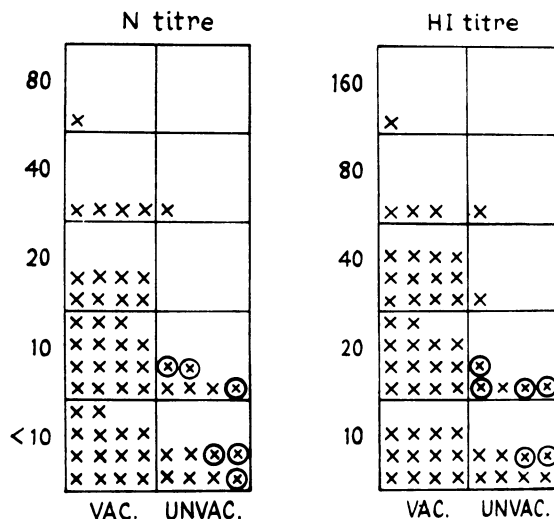


Fig. 1. HI and neutralizing (N) antibody titres of the 57 contacts (shown by crosses). Circles round the crosses denote contacts who developed smallpox.

Table 1. Antibody titres and other details of contacts of smallpox cases

Serial No. of contact	Age (years) and sex	Primary vaccination	Living in same room as index case or not	Type of index case ^a	Duration of contact (days)	Antibody titre ^b		Whether developed smallpox; if so, number of days after onset of index case, and type of secondary case ^a
						HI	Neutralizing	
1	35/M	-	Yes	C	3	80	40	No
2	40/F	-	Yes	C	3	40	10	No
3	23/M	-	No	C	3	10	< 10	No
4	38/M	+	Yes	C	7	10	10	No
5	60/M	+	Yes	C	7	40	10	No
6	24/M	+	Yes	H	2	20	10	No
7	25/M	+	Yes	H	2	20	10	No
8	17/F	+	Yes	D	8	20	10	No
9	25/F	-	Yes	D	8	10	10	No
10	18/M	+	Yes	D	8	10	< 10	No
11	13/F	-	Yes	D	8	20	10	Yes; 13 days; D
12	14/F	+	No	D	8	10	< 10	No
13	29/F	+	No	D	8	40	20	No
14	40/M	+	Yes	D	5	20	10	No
15	30/F	-	Yes	D	5	10	< 10	No
16	30/F	-	Yes	D	5	10	10	No
17	40/F	+	No	D	5	20	10	No
18	13/F	+	No	D	5	80	40	No
19	25/M	+	Yes	D	7	40	40	No
20	29/M	+	No	D	7	40	10	No
21	70/F	+	No	D	7	10	< 10	No
22	17/F	+	No	D	7	40	10	No
23	29/M	+	No	H	2	10	< 10	No
24	18/M	-	No	D	6	10	< 10	No
25	23/M	+	No	D	6	20	10	No
26	45/F	+	No	D	6	80	40	No
27	18/M	+	Yes	C	7	20	10	No
28	21/M	+	Yes	C	7	20	< 10	No
29	30/F	-	Yes	C	4	20	< 10	No
30	22/F	-	Yes	C	4	10	< 10	No
31	35/M	+	No	C	4	20	< 10	No
32	4/M	+	Yes	D	5	40	20	No
33	35/M	-	Yes	D	6	20	10	Yes; 13 days; D
34	50/M	-	No	D	6	20	< 10	Yes; 11 days; D

^a H = haemorrhagic, C = confluent, and D = discrete.

^b Expressed as reciprocal of highest dilution of serum inhibiting haemagglutination or pock formation. In the neutralizing test, the comparable titre for the international standard antismallpox serum was 320.

Table 1. Antibody titres and other details of contacts of smallpox cases (*continued*)

Serial No. of contact	Age (years) and sex	Primary vaccination	Living in same room as index case or not	Type of index case ^a	Duration of contact (days)	Antibody titre ^b		Whether developed smallpox; if so, number of days after onset of index case, and type of secondary case ^a
						HI	Neutralizing	
35	25/M	+	No	D	11	10	< 10	No
36	30/M	+	No	D	11	160	80	No
37	6/F	+	No	D	11	10	< 10	No
38	5/F	+	No	D	11	20	< 10	No
39	11/F	+	Yes	D	6	10	< 10	No
40	12/M	+	Yes	D	6	20	< 10	No
41	31/M	+	Yes	C	5	10	< 10	No
42	14/M	+	No	D	5	80	20	No
43	29/M	+	No	D	6	40	20	No
44	38/M	+	No	D	6	10	< 10	No
45	17/M	+	Yes	D	8	20	20	No
46	16/F	-	No	C	5	20	10	Yes; 10 days; D
47	25/M	+	No	D	7	20	10	No
48	28/M	-	Yes	H	3	10	< 10	Yes; 7 days; D
49	30/F	+	No	D	7	40	40	No
50	38/F	+	No	C	5	40	20	No
51	40/M	+	No	H	2	10	10	No
52	35/M	+	No	D	8	40	20	No
53	13/M	+	Yes	D	7	10	< 10	No
54	30/F	+	No	C	5	40	20	No
55	24/M	+	No	H	3	40	10	No
56	27/F	-	Yes	D	7	10	< 10	Yes; 12 days; D
57	25/M	+	No	C	5	20	10	No

ACKNOWLEDGEMENTS

The authors are indebted to Dr D. A. Henderson and Dr I. Arita of the Smallpox Eradication Unit, World Health Organization, Geneva, Switzerland, for their advice, help and constructive criticism; to the UNICEF East India Office for the loan of a vehicle to carry out the field work; and to Mr A. Pal for technical assistance.

RÉSUMÉ

NIVEAU MINIMAL D'ANTICORPS NÉCESSAIRE POUR CONFÉRER UNE PROTECTION CONTRE LA VARIOLE

Afin de déterminer approximativement le titre minimal d'anticorps nécessaire pour conférer une protection contre la variole, des échantillons de sang ont été prélevés

sur 57 contacts de 6 cas de référence pour la recherche d'anticorps inhibant l'hémagglutination (HI) et d'anticorps neutralisants (N). Six de ces contacts ont contracté

la variole. Ces 6 personnes n'avaient pas été vaccinées et présentaient des titres d'anticorps N égaux ou inférieurs à 10. Toutefois, 6 autres contacts non vaccinés présentant des titres d'anticorps N égaux ou inférieurs à 10 n'ont pas contracté la maladie. Tous les contacts

chez lesquels les titres d'anticorps N étaient égaux ou supérieurs à 20 sont restés indemnes, mais les contacts vaccinés chez lesquels la recherche d'anticorps N est restée négative n'ont pas non plus contracté la maladie.

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